Cytogenetic and molecular profiling of spotted snake head fish *Channa punctatus* (Bloch, 1793) from three Different places (Dakshineswar, Barrackpore, Dhapa) of west Bengal, India

Project report submitted to Sister Nivedita University

in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN BIOTECHNOLOGY

Submitted By
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Registration ID: 230020200495
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CERTIFICATE FROM THE SUPERVISOR

This is to certify that the project report entitled **"Cytogenetic and molecular profiling of spotted snake-head fish *Channa punctatus* (Bloch, 1793) from three Different places (Dakshineswar, Barrackpore & Dhapa) of West Bengal, India"**, submitted for the degree of M.Sc in Biotechnology by **Ms. Sneha Chatterjee, Registration ID: 230020200495; Enrolment ID: 2322210002020**, is an original research work carried out by her during the period from **January, 2025 to May, 2025** under my guidance and supervision. Further, this work has not been submitted elsewhere for the award of any degree or any type of publication.

I wish her all the best and a successful bright future.

Signature of Supervisor with Stamp

Arpita Rakshit. 20/05/2025

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "Cytogenetic and molecular profiling of spotted snake head fish *Channa punctatus* (Bloch, 1793) from three Different places (Dakshineswar, Barrackpore, Dhapa) of west Bengal, India " in partial fulfilment of the requirements for the award of the degree of Masters of science in Biotechnology is an authentic record of my own work carried out during the period from January to May under the supervision of Dr. Arpita Rakshit Department of Assistant Professor &HOD, Department of Zoology Seth Anandram Jaipuria College (under University of Calcutta)

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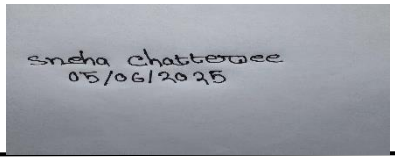

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TO WHOM IT MAY CONCERN

This is to certify that the project report titled "**Cytogenetic and molecular profiling of spotted snake head fish *Channa punctatus* (Bloch, 1793) from three Different places (Dakshineswar, Barrackpore, Dhapa) of west Bengal, India** ", submitted by Ms Sneha Chatterjee Registration ID: 230020200495, Enrolment ID: 2322210002020 to the Department of Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by her for the partial fulfilment of the requirements for the Master of Science degree.

Date: 03.06.2025

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This dissertation, entitled “Cytogenetic and molecular profiling of spotted snake head fish *Channa punctatus* (Bloch, 1793) from three Different places (Dakshineswar, Barrackpore, Dhapa) of west Bengal, India”, through the result of her effort, however, a subject of such a complicated nature could not have been addressed without the intellectual sights.

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In conclusion, this dissertation is dedicated to all those who were directly or indirectly involved, and to their unfailing love and support.

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Abbreviations

Abbreviation	Full Form
NOR	Nucleolar Organizer Region
RAPD	Random Amplified Polymorphic DNA
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
bp	Base Pair
µL	Microlitre
m	Metacentric
sm	Submetacentric
a	Acrocentric
st	Sub telocentric
FN	Fundamental Arm Number
DPX	Distrene Plasticizer Xylene (mounting medium)
TE Buffer	Tris-EDTA Buffer
SDS	Sodium Dodecyl Sulfate
EDTA	Ethylenediaminetetraacetic Acid
TAE Buffer	Tris-acetate-EDTA Buffer
UV	Ultraviolet
PEV	Position Effect Variegation
CF	Centric Fusion
CENP-B	Centromere Protein B
mM	Millimolar
rpm	Revolutions per minute
°C	Degrees Celsius
Ni	Nickel
Zn	Zinc
Cu	Copper
Pb	Lead
Cr	Chromium
Cd	Cadmium

Abstract

Channa punctatus, a freshwater fish of considerable ecological and economic importance in South Asia, was cytogenetically and molecularly analysed to assess the impact of urban pollution on its genome. Samples were collected from three environmentally stressed sites in West Bengal—Barrackpore, Dakshineswar, and Dhapa—each characterized by distinct sources of anthropogenic contamination, including industrial effluents, plastic waste, and landfill leachates.

Cytogenetic investigations using colchicine-arrested metaphase spreads revealed a stable diploid number of 2n = 34, with a karyotype formula of 16 metacentric, 14 submetacentric, and 4 acrocentric chromosomes. NORs, detected via silver nitrate staining, were located proximally on a submetacentric pair, suggesting active ribosomal gene sites. However, divergence from previously reported karyotypic patterns indicates chromosomal polymorphism possibly driven by structural rearrangements like pericentric inversions and centric fusions.

Complementing the chromosomal analysis, RAPD-PCR using the OPA-2 primer demonstrated distinct polymorphic banding profiles in samples from polluted habitats, indicating genome-level instability and mutagenic effects of environmental stressors. The amplified fragment patterns revealed gains and losses of DNA bands compared to control specimens, implicating pollution-induced genetic differentiation.

This integrative study underscores the utility of combining classical cytogenetics and RAPD-based molecular markers in understanding genome responses to environmental pressures. The findings highlight the evolutionary plasticity of *C. punctatus* and the need for ongoing biomonitoring in deteriorating freshwater habitats.

Keyword: Channa punctatus**,** Cytogenetics**,** Karyotype analysis**,** Random Amplified Polymorphic DNA (RAPD)**,** Environmental genotoxicity**,** Chromosomal polymorphism

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Introduction

Channa punctatus, commonly known as the spotted snakehead, is a freshwater teleost widely distributed throughout South and Southeast Asia. It belongs to the family Channidae, order Channiformes, and is recognized for its ecological adaptability and economic value [30]. A key morphological adaptation of this species is the presence of accessory respiratory organs, which allows it to breathe atmospheric oxygen and survive in oxygen-deficient environments such as stagnant ponds, floodplains, and swamps [30].

From an ecological perspective, *C. punctatus* serves as an apex predator in many freshwater ecosystems. Its carnivorous feeding behaviour, targeting small fish and invertebrates, plays a regulatory role in population control and ecological balance. Economically, it is highly valued as a food fish, with additional applications in ethnomedicine, particularly in aiding the recovery of patients due to its high protein content and traditional medicinal significance [30], [31].

1.1 Importance of Cytogenetic Studies in Fish

Cytogenetic studies—especially karyotyping and nucleolar organizer region (NOR) analysis—are essential tools in fish genetics. They provide insights into the evolution, systematics, population structure, and species differentiation of aquatic organisms [14], [13]. These studies are also instrumental in:

- Aquaculture improvement through genetic monitoring and selective breeding,
- Conservation biology via detection of intraspecific variability,
- Chromosomal evolution and speciation research,
- And in resolving taxonomic ambiguities in morphologically similar species [1], [14], [13].

However, cytogenetic analysis in fish is challenging due to the high number of small-sized chromosomes, which limits the clarity of metaphase spreads and often requires specialized techniques for effective visualization [20].

1.2 Role of Nucleolar Organizer Regions (NORs)

NORs are chromosomal regions that house genes coding for 18S and 28S ribosomal RNA, essential components of protein synthesis. These regions can be visualized using silver staining techniques, which specifically bind to argyrophilic proteins in the nucleolus [17], [18]. The analysis of NORs aids in identifying:

- Chromosomal polymorphisms between and within species,
- Karyotypic evolutionary patterns, and
- Population-level genetic markers useful in taxonomy and evolutionary biology [16], [18].

In ichthyology, NORs are considered important cytogenetic markers, especially for understanding the genetic consequences of pollution, geographic isolation, and adaptive divergence [18], [32].

1.3 Molecular Tools: RAPD as a Genetic Marker

Random Amplified Polymorphic DNA (RAPD) is a PCR-based technique that uses arbitrary short primers to amplify random segments of the genome, making it a powerful tool for detecting genetic variation without prior genome knowledge [16], [23]. This method has several advantages:

- It requires small quantities of DNA,
- It is cost-effective and rapid,
- It exhibits high polymorphism, and
- It is not influenced by environmental conditions [16], [23].

RAPD has been widely applied in studies on population genetics, stock differentiation, and hybrid detection in fishes such as tilapia, common carp, guppy, Indian major carps, and others [2], [3], [4], [21], [26]. Despite its advantages, RAPD can suffer from reproducibility issues, and interpretation may be subjective due to band intensity variability [9], [22].

1.4 Relevance of Studying Indigenous Species

Research on native species like *Channa punctatus* in localized environments, especially in polluted urban water systems, is still scarce. Region-specific cytogenetic and molecular analyses are crucial for:

- Understanding intraspecific variation caused by pollution and environmental stress,
- Supporting biodiversity conservation efforts,
- Revealing chromosomal polymorphisms that might underlie adaptive traits [13], [29].

Environmental pressures such as industrial discharge, agricultural runoff, and urban waste accumulation contribute to genotoxicity, which may alter gene expression or induce chromosomal damage [32]. These changes can limit gene flow, affect reproduction, and ultimately lead to loss of genetic diversity or even local extinction [29], [32].

The Specimen: ***Channa punctatus***

Figure 1: ***Channa punctatus***

5.1 Nomenclature and Taxonomy

- **Scientific name:** *Channa punctatus*
- **Common name:** Spotted snakehead
- **Local names:** Lata, Taki, Okol
- **Taxonomic classification:**
  - **Phylum:** Chordata
  - **Class:** Osteichthyes
  - **Order:** Channiformes
  - **Family:** Channidae
  - **Genus:** *Channa*
- **Historical Synonyms:**
  - *Ophicephalus punctatus* Bloch, 1794 [8]
  - *Ophicephalus punctatus* Day, 1878 [10]
  - *Ophicephalus punctatus* Shaw and Shebbeare, 1937 [58]
  - *Ophicephalus punctatus* Bhuiyan, 1964 [7]

5.2 Morphological Description

- **Fin formula:** Dorsal (D) 29–32; Pectoral (P_1) 15–18; Pelvic (P_2) 6; Anal (A) 20–22 [30].
- **Head length** is 3.2–3.5 times the total length and approximately 2.7 times the standard length [31].
- **Eyes** are small and situated toward the anterior of the head.
- **Two pairs of nostrils** occur near the superior-anterior angle of the eyes.
- **Lower jaw** is slightly protruding and houses conical teeth.
- **Barbels** are absent, which differentiates the species from certain other freshwater teleost.
- **Scales** are large and irregularly arranged on the head.
- The **pectoral fin** is positioned just above the pelvic fin, while the caudal fin is long and rounded.

- **Body coloration** in live individuals is typically brown dorsally, fading ventrally with no evident spotting or striations [30], [31].

Size: Bhuiyan (1964) documented a maximum length of 30 cm for *C. punctatus* [7], whereas Rahman (1989) reported specimens exceeding 240 mm in total length [31].

5.3 Habit and Habitat

According to Bhatti (1934), *Channa punctatus* is well-adapted to low-oxygen freshwater habitats, particularly muddy and stagnant waters, such as floodplains, ditches, and weedy ponds [6]. It can burrow into mud, construct nests from floating weeds, and even move across land between pools during seasonal droughts. It is a voracious carnivore, preying on small fish, invertebrates, and fish larvae, playing a pivotal role in maintaining aquatic ecological balance.

5.4 Economic Importance

The species holds significant commercial and nutritional value across South Asia. Its firm white flesh, widespread availability, and low-cost capture make it a popular edible species, especially among rural and semi-urban populations [30]. Moreover, *C. punctatus* is traditionally believed to aid recovery in ill patients and is sometimes included in ethnomedicinal formulations.

5.5 Status and Conservation

While *C. punctatus* is not currently classified as threatened by the IUCN, local populations face environmental stress due to the following:

- **Agricultural runoff** containing pesticides and fertilizers,
- **Industrial effluents** containing heavy metals,
- Urban domestic waste and sewage.

Study Area

In the present investigation, specimens of *Channa punctatus* were collected from three heavily polluted urban water bodies in West Bengal: Barrackpore, Dakshineswar, and Dhapa. These locations were selected for their ecological significance as well as their chronic exposure to anthropogenic pollution, making them ideal for evaluating the cytogenetic and molecular responses of local fish populations.

2.1 Barrackpore

Located on the banks of the Hooghly River, Barrackpore is a densely populated suburban area situated north of Kolkata. The sampling site is directly impacted by effluents from jute,

textile, and chemical industries, which discharge untreated wastewater into nearby ponds and canals. These industrial activities have been associated with high concentrations of heavy metals, particularly cadmium (Cd), chromium (Cr), and lead (Pb) in aquatic environments. These metals accumulate in the gills and liver tissues of fish, where they induce chromosomal aberrations such as chromatid breaks, centromeric gaps, deletions, and fragmentation [32].

2.2 Dakshineswar

The Dakshineswar site is situated near a temple complex along the Hooghly River, where a polluted urban nala (drain) channels domestic waste, plastic materials, and sewage into adjacent water bodies. The accumulation of plastic waste—including discarded packets, bottles, and food wrappers—alongside household refuse, contributes to chemical leaching, particularly of Pb, Cd, Cr, and Cu. Although direct heavy metal assessments from Dakshineswar are limited, similar environments have shown that such microplastic-associated pollution can cause bioaccumulation of metals in aquatic organisms, leading to genotoxic stress and reduced genetic stability [29], [32].

2.3 Dhapa

Dhapa, a sprawling landfill site on the eastern fringes of Kolkata, serves as the city's primary municipal dumping ground. The site is infamous for untreated leachate, solid waste seepage, and sewage-fed agriculture. This has led to alarming concentrations of heavy metals such as Cd, Cr, Pb, zinc (Zn), copper (Cu), and nickel (Ni) in the surrounding soil and water. These pollutants find their way into local aquatic ecosystems and agriculture, impacting organisms at multiple trophic levels. Several studies have demonstrated that organisms residing near Dhapa show significant chromosomal abnormalities, including those linked to heavy metal-induced mutagenesis [29], [32].

Figure 2: Polluted water bodies in West Bengal—Barrackpore, Dakshineswar, and Dhapa—sampling sites for *Channa punctatus* cytogenetic study.

Table 1: study area with source of pollution and it affects

Location	Pollution Source	Heavy Metals Detected	Cytogenetic Effects on *Channa punctatus*
Barrackpore	Industrial effluents (jute, textile, chemical sectors)	Cd, Cr, Pb	Chromosomal aberrations: centromere gaps, chromatid breaks, deletions, etc.
Dakshineswar	Domestic plastic waste (packets, bottles, household garbage)	Pb, Cd, Cr, Cu	Heavy metal accumulation leading to genotoxic effects in comparable environments
Dhapa	Landfill leachate, sewage-fed agriculture	Cd, Cr, Pb, Zn, Cu, Ni	High metal accumulation in soil; genotoxic effects in exposed organisms

Materials and Methods

This study utilized a dual approach involving cytogenetic analysis (chromosomal studies and silver nitrate staining of NORs) and molecular profiling using Random Amplified Polymorphic DNA (RAPD) markers. Both methodologies were employed to evaluate chromosomal architecture and DNA polymorphism in *Channa punctatus* specimens collected from different polluted habitats in West Bengal.

3.1 Cytogenetic Preparation and Karyotype Analysis

Live specimens of *Channa punctatus* were collected from Barrackpore, Dakshineswar, and Dhapa and acclimatized under laboratory conditions. For cytogenetic study, 1% colchicine solution was injected intraperitoneally at a dosage of 1 mL per 100 grams of body weight to arrest cells at metaphase [19]. After a 4–5-hour exposure period, the fish were euthanized, and tissue samples were extracted from gill arches and head kidney, which are known to have a high mitotic index [20].

Tissues were mechanically homogenized using cold hypotonic 1% trisodium citrate solution, and the suspension was incubated for 30 to 60 minutes at room temperature to achieve optimal cell swelling. Following this, the suspension was transferred to centrifuge tubes and spun at 2,000 rpm for 10 minutes. The supernatant was discarded, and the resulting pellet was fixed using a mixture of glacial acetic acid and methanol in a 1:3 ratio. The fixative treatment was repeated three times, with centrifugation steps in between, to improve chromosomal spreading and cellular integrity [20], [19].

Fixed cells were dropped onto pre-cooled glass slides and passed briefly through a spirit flame to enhance spreading. Slides were then air-dried thoroughly.

To detect nucleolar organizer regions (NORs), silver nitrate staining was carried out following the method of Howell and Black (1980) [16]. A solution composed of freshly prepared colloidal developer and silver nitrate (1:2 ratio) was applied, and the slides were incubated at 60°C in a dark humidified chamber. The NORs appeared as dark black dots against a light chromosomal background due to argyrophilic protein interaction [16], [17].

Following NOR staining, the same slides were subjected to Giemsa staining to allow visualization of chromosome morphology. Giemsa working solution was applied for 10–15 minutes, rinsed with distilled water, air-dried, and mounted with DPX for microscopic observation.

Chromosome classification was based on centromere position, adopting the nomenclature proposed by Levan et al. (1964), categorizing chromosomes as metacentric (m), sub metacentric (sm), sub telocentric (st), or acrocentric (a) [54].

3.2 Genomic DNA Extraction and Purity Evaluation

For molecular analysis, genomic DNA was extracted from scales using the modified protocol of Wasko et al. (2003) [20]. Approximately 50 mg of dried scale tissue was excised, finely chopped, and transferred into a 2 mL Eppendorf tube containing:

- 940 µL lysis buffer (200 mM Tris-HCl pH 8, 100 mM EDTA, 250 mM NaCl),
- 30 µL of Proteinase K, and
- 30 µL of 20% SDS.

The sample was incubated at 48°C for 45–50 minutes in a water bath to allow thorough digestion of proteins and cellular debris. Following digestion, an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and gently inverted for 10 minutes to precipitate proteins and separate nucleic acid layers.

After centrifugation at 9,200×g for 10 minutes, the aqueous phase was transferred to a clean tube. DNA was precipitated by adding isopropanol and 0.2 volumes of 10 M ammonium acetate, followed by slow inversion. The resulting DNA pellet was collected by centrifugation at 13,200×g for 10 minutes, washed with 500 µL of chilled 70% ethanol, air-dried, and rehydrated in 200 µL TE buffer.

Purity assessment of DNA was performed by spectrophotometric measurement at 260/280 nm. DNA integrity was checked by gel electrophoresis on 0.7% agarose gel stained with ethidium bromide, visualized under UV light.

3.3 RAPD-PCR Amplification and Electrophoresis

PCR amplification of genomic DNA was performed using OPA-2 primer (5′–CATTCCGGCC–3′), commercially synthesized by IDT (Integrated DNA Technologies). The reaction mixture (total 50 μL) consisted of:

- 10× PCR buffer with 50 mM KCl,
- 2 mM $MgCl_2$,
- 0.2 mM of each dNTP,
- 1 μM of OPA-2 primer,
- 1 U of Taq polymerase, and
- 20–50 ng of template DNA [21].

The PCR program included:

- Initial denaturation: 94°C for 1 minute,
- Annealing: 55°C for 1 minute,
- Extension: 72°C for 2 minutes,
- Repeated for 30 cycles, followed by a final extension at 72°C for 5 minutes and storage at 4°C.

PCR products were separated by agarose gel electrophoresis using 1% gel in 1× TAE buffer, supplemented with ethidium bromide. The gel was run at 70–80 volts, and a 100–2000 bp DNA ladder was used to determine fragment size.

Only clear, reproducible bands present across multiple replicates were considered valid for genetic analysis. Differences in band intensity were not used in scoring, as recommended in previous RAPD-based fish studies [21], [22], [23].

Result And Discission

Chromosome Number and Karyotypic Composition of *Channa punctatus*: Evidence of Intraspecific Variation

The diploid chromosome number (2n) in *Channa punctatus*, a widely distributed freshwater teleost, has been established as 32 in the present investigation. The karyotype is composed of 16 metacentric (m), 14 sub metacentric (sm), and 2 acrocentric (a) chromosomes, resulting in a karyotypic formula (KF) of 16m + 14sm + 2a. Based on the morphology of the chromosomes and their arm structures, the Fundamental Arm Number (FN)—which accounts for the number of chromosomal arms—is calculated to be 52. This configuration is typical of a fairly symmetrical karyotype, indicating chromosomal stability in this population [30].

The population of *C. punctatus* examined from Singur, West Bengal, consistently exhibited this chromosomal pattern across multiple metaphase spreads, confirming the diploid number of 32 chromosomes. Importantly, one pair of submetacentric chromosomes was observed to carry nucleolar organizer regions (NORs) with strong silver nitrate staining. These NORs

were located proximally near the centromeric region, suggesting a highly active rDNA gene cluster in this chromosomal location. NORs are often associated with ribosomal RNA gene sites, and their position and activity can be significant markers for taxonomic and evolutionary studies [18], [16].

However, this present chromosomal profile contrasts with earlier findings by Manna (1983), who reported a distinctly different karyotypic composition in *C. punctatus*. According to that study, the species exhibited a karyotype with 14 metacentric, 16 sub telocentric, and 4 acrocentric chromosomes [13]. Such a variation in the chromosomal arrangement points toward the presence of intraspecific chromosomal polymorphism, which may arise through various mechanisms such as centric fusion, pericentric inversion, or other structural rearrangements. This discrepancy may also reflect localized population-level karyotypic differentiation or methodological differences in chromosomal classification [29].

The observed divergence in karyotypic formula could be indicative of chromosomal evolution within *C. punctatus* populations, potentially driven by geographic isolation, ecological pressures, or chromosomal rearrangements that affect centromere position and chromosome morphology. Structural changes like pericentric inversion can alter the relative position of the centromere, thereby converting sub telocentric chromosomes into metacentric or submetacentric forms without altering the chromosome number [29]. Similarly, centric fusion (Robertsonian translocation) can reduce the number of acrocentric chromosomes by fusing them into bi-armed chromosomes, further modifying the karyotypic pattern [32].

Such karyotypic plasticity within a single species not only provides insight into the dynamics of chromosome evolution in fishes but also suggests the potential role of chromosomal rearrangements in adaptive divergence and speciation. The NOR-bearing chromosomes, in particular, can be valuable cytogenetic markers for identifying chromosomal polymorphisms and tracking evolutionary change within and across populations [18], [29].

In conclusion, the current study of *C. punctatus* from Singur reveals a diploid chromosome number of 2n = 32 with a predominance of metacentric and submetacentric chromosomes, alongside two acrocentric, differing notably from earlier reports. This emphasizes the significance of continued cytogenetic monitoring across different populations to better understand the chromosomal variability, evolutionary trends, and potential taxonomic complexities within this species

Pericentric Inversion and Its Role in the Chromosomal Evolution of *Channa* Species

Pericentric inversion is a chromosomal rearrangement characterized by the breakage of a chromosome in two places, including the centromere, followed by the reinsertion of the inverted segment into the chromosome. Importantly, this rearrangement alters the order of genes within the chromosome segment but does not change the total chromosome number in the organism. This feature distinguishes pericentric inversions from other types of

chromosomal changes such as fusions or fissions, which can alter chromosome counts. In the *Channa* genus, commonly known as snakehead fishes, extensive karyotypic studies have revealed chromosome polymorphisms, particularly involving sub telocentric and acrocentric chromosome types, which strongly suggest that pericentric inversions have been a driving force in their karyotype evolution [29].

The occurrence of such inversions is evident in the diversity of chromosomal forms within populations, indicating ongoing chromosomal rearrangements that contribute to genetic differentiation. The shifts between sub telocentric and acrocentric chromosomes in *Channa* species are a hallmark of pericentric inversion events, whereby the position of the centromere shifts along the chromosome, leading to a change in chromosome morphology without altering chromosome number. This phenomenon provides an effective mechanism for generating genetic variability while maintaining genome stability at the level of chromosome count [29].

At the molecular level, recent advances in genetics help explain how pericentric inversions influence speciation processes. One crucial effect is the induction of position effect variegation (PEV), a phenomenon whereby genes near heterochromatic regions—typically gene-poor, tightly packed chromatin surrounding the centromere—experience variable expression depending on their chromosomal context. Chromosomal rearrangements that reposition pericentric heterochromatin next to euchromatin can cause such heritable changes in gene activity. This variegation can result in the silencing or altered expression of genes that normally reside in euchromatic, transcriptionally active regions, as well as genes within heterochromatin itself [45], [46], [47].

This repositioning and modification of chromatin structure can lead to diverse phenotypic outcomes, potentially influencing fitness and adaptability. In *Channa* species, these genetic and epigenetic changes may help drive divergence between populations by promoting differential gene expression, which could eventually contribute to reproductive isolation [47].

Moreover, telomeric regions, which cap chromosome ends, have been shown to exert long-range position effects influencing gene transcription at considerable distances from the telomere itself [48]. These telomeric effects might affect genes located on the short arm of the inverted chromosome, whereas heterochromatic position effects predominate in the long arm. Thus, pericentric inversions can create a complex landscape of gene regulation through chromosomal context, affecting multiple genomic regions differently.

Another critical consequence of pericentric inversion is its impact on meiotic recombination. During meiosis, inverted chromosome segments suppress recombination within the inverted region because crossing over can produce unbalanced gametes that reduce fertility. This suppression effectively locks together groups of genes within the inverted segment, preserving specific gene combinations that may provide selective advantages to the organism. These linked gene clusters can act as “supergenes,” facilitating adaptation to specific

environments or ecological niches. In *Channa*, this mechanism might maintain advantageous traits in populations, promoting divergence and speciation [29], [47].

Centric Fusion and Chromosomal Evolution in *Channa* Species

Centric fusion (CF), also referred to as Robertsonian translocation, is a chromosomal rearrangement wherein two acrocentric or telocentric chromosomes become joined at or near their centromeres, forming a single metacentric chromosome. Unlike pericentric inversion, CF results in a reduction in chromosome number while generally preserving the total amount of genetic material. In the context of *Channa* species, comparative cytogenetic analyses have revealed noticeable decreases in diploid chromosome number accompanied by a relative increase in the number of metacentric chromosomes. This pattern strongly indicates that CF has played a significant role in shaping the karyotype structure within this genus during its evolutionary history [29].

Empirical evidence supports this hypothesis. For instance, *Channa punctatus* has been reported to exhibit two distinct karyotypic forms: one with a diploid number of 2n = 34, comprising 16 metacentric (m), 14 submetacentric (sm), and 4 acrocentric (a) [31], and another with 2n = 32, composed of 16m + 14sm + 2a (present study). The apparent reduction of acrocentric from four to two, with a corresponding rise in submetacentric chromosomes, suggests that centric fusion events have occurred, converting two acrocentric into a single submetacentric. Such alterations reinforce the idea that CF is an ongoing mechanism contributing to the dynamic karyotypic evolution within *Channa* populations [29], [31].

Despite its cytogenetic relevance, the molecular mechanisms underpinning centric fusion events remain largely obscure. To date, no conclusive experimental evidence has uncovered the exact DNA-level processes that drive CF in vertebrate genomes, including in fishes. However, some molecular insights have emerged from studies in mammals. Garagna et al. (2001) proposed that a specific complex involving minor satellite DNA sequences and the centromere-binding protein CENP-B may serve as the molecular scaffold that facilitates centric fusion [50]. This model suggests that CF may not require large-scale chromosomal reorganization, but rather subtle structural shifts at the centromeric region that permit the stable joining of two chromosomes.

Nonetheless, evidence for major DNA loss during CF remains elusive. Investigations involving house mice, which can carry up to 18 Robertsonian fusions, have failed to detect significant DNA loss associated with these rearrangements [49]. These findings challenge the notion that chromosomal fusion must involve deletion of genetic material and support the hypothesis that CF may be primarily a structural change, rather than one driven by sequence-level modification.

Another intriguing aspect of CF in *Channa* is its potential contribution to speciation, although the molecular basis for this process is not fully understood. The formation of Robertsonian fusions can disrupt normal chromosomal pairing during meiosis, especially in heterozygous

individuals, leading to reduced fertility or reproductive barriers. Over evolutionary timescales, such chromosomal incompatibilities can promote reproductive isolation, a fundamental step toward the emergence of new species. However, in contrast to the more thoroughly studied effects of chromosomal inversions, the role of CF in initiating or maintaining reproductive isolation in fishes remains speculative. This is due in part to the lack of comprehensive molecular and genomic data detailing how such fusions alter gene expression or influence hybrid viability [29], [31].

Centric fusion appears to be a critical driver of karyotypic evolution within *Channa* species, leading to a decrease in chromosome number while reshaping chromosomal morphology. Though the precise molecular events responsible for CF remain to be fully elucidated, existing cytogenetic evidence, coupled with emerging molecular models, provides a framework for understanding how these rearrangements may influence evolutionary divergence. Further studies integrating genomics, chromatin biology, and reproductive studies are essential to fully uncover the role of CF in speciation within this diverse and ecologically important group of fishes.

Differentiation of Constitutive Heterochromatin in the Evolution of *Channa* Species

Constitutive heterochromatin, which is typically composed of highly repetitive DNA sequences and remains transcriptionally inactive, plays a significant role in the structural organization of chromosomes. In the context of *Channa* species evolution, observable variations in the quantity and distribution of heterochromatin suggest that this component of the genome has undergone species-specific differentiation. A comparative analysis of C-banding patterns among three species—*Channa orientalis*, *Channa striatus*, and *Channa punctatus*—reveals that C-bands are expressed in approximately 38%, 25%, and 30% of the chromosomal complements, respectively. Notably, the C-bands in *C. orientalis* and *C. striatus* appear faint and relatively diffuse, whereas in *C. punctatus*, these bands are more prominent, both in size and density. This suggests a possible amplification of heterochromatic regions, particularly centromeric heterochromatin, in certain lineages of *Channa* [31].

These findings point to the evolutionary differentiation of centromeric heterochromatin across species, even though definitive molecular evidence is still required to confirm these cytological observations. The pattern is consistent with theoretical models of karyotypic evolution, where centromeric heterochromatin may either increase or diminish over evolutionary time. According to Hsu and Arrighi (1971), some chromosomal evolutionary pathways are characterized by the expansion of centromeric heterochromatin [53], while in others, as proposed by Yosida (1975), a reduction or erosion of these regions occurs [54]. Such divergent trajectories may explain the heterogeneity in heterochromatin content observed among *Channa* species, which likely reflects species-specific adaptive or structural genome requirements.

Although heterochromatin is traditionally viewed as genetically inert due to its paucity of protein-coding genes, changes in its quantity and distribution can have significant functional consequences. While Saito and Laemmli (1993) suggest that C-bands generally do not harbour genes [55], their influence on neighbouring euchromatic regions can be profound. The phenomenon of position effect variegation (PEV), as described by Weiler and Wakamoto (1995), demonstrates that the proximity of genes to heterochromatic regions can result in heritable changes in gene expression [48]. Therefore, quantitative variations in centromeric heterochromatin, even in the absence of direct gene content, could potentially alter the expression profiles of adjacent genes.

In the present study, although similar data are not yet available for all species of the genus *Channa*, the observed differences in centromeric C-band size across three species suggest that heterochromatic shifts might be contributing to genome evolution in a subtle yet significant manner. These changes may not induce mutations per se, due to the presumed lack of gene content in C-bands, but they may nonetheless influence chromatin structure, nuclear architecture, and gene regulation. Furthermore, since such chromatin effects are often heritable, they can contribute to phenotypic diversity and potentially to the reproductive isolation necessary for speciation [48], [55].

Taken together, the differentiation of constitutive heterochromatin in *Channa* species reflects an important, albeit underexplored, component of chromosomal evolution. While the precise molecular mechanisms behind these changes remain to be elucidated through advanced genomic and epigenomic studies, the current cytogenetic evidence provides compelling support for the evolutionary role of heterochromatin in this genus. Future investigations incorporating high-throughput sequencing, chromatin profiling, and comparative genomics are essential for understanding how these heterochromatic modifications shape the evolutionary trajectories of *Channa* fishes.

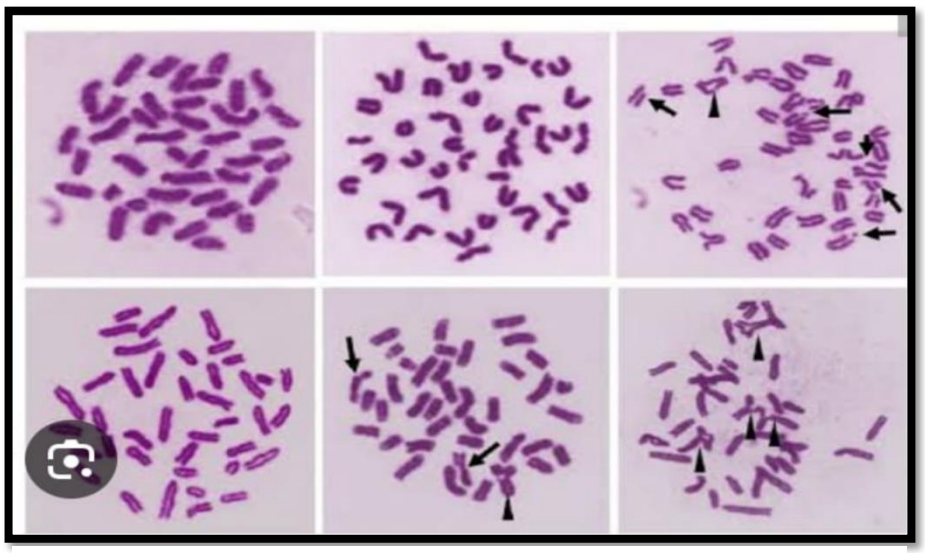

Figure 3: Metaphase spreads of *Channa punctatus* showing normal karyotypes (top left) and pollution-induced chromosomal abnormalities (arrows and arrowheads) in exposed samples

Chromosomal aberrations

Species	Karyotype	Aberration
C. punctatus	2n = 34 = 16m + 14sm + 4a	NO 1=14M+14SM+ 2B +2A NO 2 = 14M+13SM+ 3B +2A NO3 = 14M+14SM+2B NO 4 =16M+16SM METACENTRIC FUSION

Gel- Electrophoresis

Difference in band size in the study areas from the control sample indicates genomic rearrangements due to pollution. RAPD-PCR with the OPA-2 primer generates DNA fragments that reflect polymorphisms in the genome. Band differences indicate **genetic variations or mutations**, often due to environmental stress (e.g., pollutants).

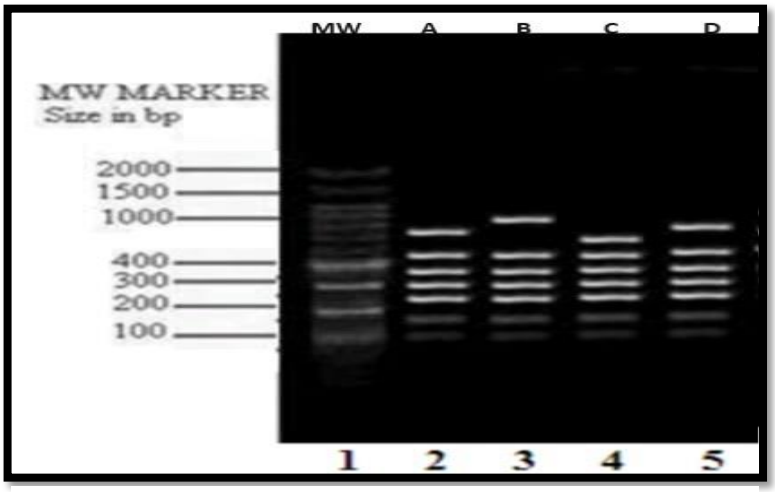

Figure 4: RAPD profiles of *Channa punctatus* with OPA-2 primer showing band variation between control and polluted samples

Sample	Banding Pattern	Interpretation
A (Lane = 2)	Clear, fewer, stable bands	Control from unpolluted site
B, C, D (Lane = 3,4,5)	Extra/missing bands, variation in band intensity	Genetic polymorphism due to pollution stress

Conclusion:

This investigation into the cytogenetic and molecular responses of *Channa punctatus* populations from heavily polluted urban water bodies in West Bengal reveals critical insights into the genetic and chromosomal consequences of environmental stress. The karyotypic assessment confirmed a consistent diploid number of 2n = 34 in the studied populations, with notable structural polymorphisms such as variations in the number and morphology of NOR-bearing chromosomes. Such chromosomal variations suggest localized genomic adaptations, possibly driven by selective pressures imposed by pollutants such as cadmium, chromium, lead, copper, and other heavy metals.

Moreover, the application of RAPD-PCR using the OPA-2 primer revealed distinct polymorphic banding patterns among populations from Barrackpore, Dakshineswar, and Dhapa when compared to reference samples. These banding differences, characterized by the appearance or disappearance of bands and variation in intensity, are indicative of underlying DNA damage, mutations, or stress-induced genomic rearrangements. This supports the hypothesis that long-term exposure to genotoxic pollutants has led to detectable genetic differentiation among these fish populations.

The study clearly establishes *Channa punctatus* as a potential bioindicator species for evaluating freshwater ecosystem health, especially in regions impacted by anthropogenic waste and industrial effluents. The integration of cytogenetic tools like NOR staining with molecular techniques such as RAPD enhances our ability to detect and monitor genotoxic effects in aquatic environments with precision.

Future Perspectives

While this study provides a foundational understanding of pollution-induced genetic variation in *C. punctatus,* further research is essential. Future investigations should include:

- **High-throughput sequencing and epigenetic profiling** to identify specific genes or regulatory elements impacted by pollution.
- **Comparative analysis with other sympatric fish species** to assess differential susceptibility and resilience to genotoxic stress.
- **Longitudinal studies** to monitor temporal changes in genetic structure and karyotypic stability under ongoing environmental degradation.
- **Development of biomarker panels** using RAPD or other marker systems for rapid environmental assessment.

Such approaches will enhance conservation strategies and support the formulation of environmental regulations. Ultimately, cytogenetic and molecular biomonitoring can

contribute to a sustainable framework for aquatic biodiversity preservation, especially in urban and industrially affected habitats

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